

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Withdrawn) A process for preparing a variant of Erysipelothrix rhusiopathiae surface protective antigen SpaA protein or of a shortened form thereof ΔSpaA protein in which a portion of SpaA protein is deleted, said variant having immunogenicity and being expressed in E. coli as inclusion bodies, which comprises mutating a gene coding for said SpaA or ΔSpaA protein so that amino acid substitution may be introduced in the amino acid sequence of said SpaA or ΔSpaA protein, allowing the resulting mutated gene to be expressed in E. coli, and selecting such a variant that formed inclusion bodies among the variants expressed.

2. (Withdrawn) The process of claim 1 which comprises the following steps (A) to (D):

(A) introducing mutation in a gene coding for soluble Erysipelothrix rhusiopathiae surface protective antigen SpaA or ΔSpaA protein so that amino acid substitution may be introduced;

(B) transforming E. coli cells with an expression vector containing the resulting mutated gene;

(C) selecting E. coli cells that formed insoluble inclusion bodies among the above transformed E. coli cells; and

(D) culturing the selected E. coli cells for recovery of the inclusion bodies within the cells.

3. (Withdrawn) The process of claim 2 which after step (D) further comprises the following steps (E) to (F):

(E) administering the inclusion bodies or the inclusion bodies treated with a solubilizing agent to an animal sensitive to Erysipelothrix rhusiopathiae infection and then attacking said animal with a virulent strain of Erysipelothrix rhusiopathiae; and

(F) observing survival or death of the animal sensitive to Erysipelothrix rhusiopathiae to thereby assess the presence of a protective activity (immunogenicity) against Erysipelothrix rhusiopathiae infection.

4. (Withdrawn) The process of any one of claims 1 to 3 wherein said amino acid substitution is one or a combination of more than one selected from the group consisting of (1) to (7) as described below:

(1) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(2) the 154th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(3) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;

(4) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;

(5) the 253rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;

(6) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

and

(7) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine.

5. (Withdrawn) The process of any one of claims 1 to 3 wherein said amino acid substitution is one selected from the group consisting of (a) to (h) as described below:

(a) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(b) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;

(c) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;

(d) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(e) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(f) the 154th and 203rd amino acids from the N-terminal encompassing the signal sequence are substituted with glycine and threonine, respectively;

(g) the 214th and 253rd amino acids from the N-terminal encompassing the signal sequence are substituted with glutamine and threonine, respectively; and

(h) the 69th, 154th and 203rd amino acids from the N-terminal encompassing the signal sequence are substituted with glycine, glycine and threonine, respectively.

6. (Withdrawn) The process of any one of claims 1 to 3 wherein said Erysipelothrix rhusiopathiae is selected from the group consisting of Fujisawa strain, Koganai strain, Tama 96 strain, SE-9 strain and Shizuoka 63 strain.

7. (Withdrawn) The process of any one of claims 1 to 6 wherein SpaA or Δ SpaA protein before introduction of said amino acid substitution has the amino acid sequence as depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2 with deletion at its C-terminal, respectively.

8. (Currently Amended) An isolated variant of an Erysipelothrix rhusiopathiae surface protective antigen SpaA protein or of a shortened form thereof (known as Δ SpaA protein),

wherein the SpaA protein has the amino acid sequence of SEQ ID NO: 2, and the Δ SpaA protein ~~which~~ is a shortened

form of the SpaA protein in which a portion about one third of the C-terminal of the SpaA protein is deleted,

wherein said variant is immunogenic, and expressed in E. coli as inclusion bodies, and has an amino acid sequence with an amino acid substitution consisting of one or a combination of more than one is selected from the group consisting of: (1) to (7) as described below:

- (1) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (2) the 154th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (3) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (4) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;
- (5) the 253rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (6) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine; and
- (7) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine.

(1) the SpaA protein comprising the amino acid substitution at position 531 (arginine to glycine);

(2) the SpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(3) the ΔSpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(4) the ΔSpaA protein comprising the amino acid substitutions at position 69 (glutamic acid to glycine), at position 154 (glutamic acid to glycine), and at position 203 (isoleucine to threonine); and

(5) the ΔSpaA protein comprising the amino acid substitution at position 278 (aspartic acid to glycine).

9-16. (Cancelled)

17. (Currently Amended) A composition comprising as an active ingredient an isolated variant of an Erysipelothrix rhusiopathiae surface protective antigen SpaA protein or of a shortened form thereof (known as ΔSpaA protein),

wherein the SpaA protein has the amino acid sequence of SEQ ID NO: 2, and the ΔSpaA protein which is a shortened form of the SpaA protein, in which a portion about one third of the C-terminal of the SpaA protein is deleted,

wherein said variant is immunogenic, and expressed in E. coli as inclusion bodies, and has an amino acid sequence with an amino acid substitution consisting of one or a

~~combination of more than one~~ is selected from the group consisting of: (1) to (7) as described below:

- (1) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (2) the 154th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (3) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (4) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;
- (5) the 253rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (6) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
and
- (7) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine.
- (1) the SpaA protein comprising the amino acid substitution at position 531 (arginine to glycine);
- (2) the SpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(3) the Δ SpaA protein comprising the amino acid substitutions at position 214 (histidine to glutamine) and at position 253 (methionine to threonine);

(4) the Δ SpaA protein comprising the amino acid substitutions at position 69 (glutamic acid to glycine), at position 154 (glutamic acid to glycine), and at position 203 (isoleucine to threonine); and

(5) the Δ SpaA protein comprising the amino acid substitution at position 278 (aspartic acid to glycine).

18-25. (Cancelled)

26. (Withdrawn) A gene coding for a variant of Erysipelothrix rhusiopathiae surface protective antigen SpaA or of a shortened form thereof Δ SpaA protein in which a portion of SpaA protein is deleted, said variant being immunogenic and expressed in E. coli as inclusion bodies.

27. (Withdrawn) The gene of claim 26 which codes for an amino acid sequence of SpaA or Δ SpaA protein wherein amino acid substitution is introduced.

28. (Withdrawn) The gene of claim 26 or 27 which is prepared by mutating a gene coding for SpaA or Δ SpaA protein to introduce amino acid substitution in the amino acid sequence of said SpaA or Δ SpaA protein, expressing the thus mutated gene in E. coli, and selecting among the expressed variants those that formed inclusion bodies.

29. (Withdrawn) The gene of any one of claims 26 to 28 which is prepared by the following steps (A) to (D) :

- (A) introducing mutation in a gene coding for soluble Erysipelothrix rhusiopathiae surface protective antigen SpaA or ΔSpaA protein so that amino acid substitution may be introduced;
- (B) transforming E. coli cells with an expression vector containing the resulting mutated gene;
- (C) selecting E. coli cells that formed insoluble inclusion bodies among the above transformed E. coli cells; and
- (D) culturing the selected E. coli cells for recovery of the inclusion bodies within the cells.

30. (Withdrawn) The gene of claim 29 which is prepared by the following steps (E) to (F) subsequent to step (D) :

- (E) administering the inclusion bodies or the inclusion bodies treated with a solubilizing agent to an animal sensitive to Erysipelothrix rhusiopathiae infection and then attacking said animal with a virulent strain of Erysipelothrix rhusiopathiae; and
- (F) observing survival or death of the animal sensitive to Erysipelothrix rhusiopathiae to thereby assess

the presence of a protective activity (immunogenicity) against Erysipelothrix rhusiopathiae infection.

31. (Withdrawn) The gene of any one of claims 26 to 30 wherein said amino acid substitution in said variant is one or a combination of more than one selected from the group consisting of (1) to (7) as described below:

- (1) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (2) the 154th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (3) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (4) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;
- (5) the 253rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (6) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
and
- (7) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine.

32. (Withdrawn) The gene of any one of claims 26 to 30 wherein said amino acid substitution in said variant is one

selected from the group consisting of (a) to (h) as described below:

- (a) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (b) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (c) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;
- (d) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (e) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (f) the 154th and 203rd amino acids from the N-terminal encompassing the signal sequence are substituted with glycine and threonine, respectively;
- (g) the 214th and 253rd amino acids from the N-terminal encompassing the signal sequence are substituted with glutamine and threonine, respectively; and
- (h) the 69th, 154th and 203rd amino acids from the N-terminal encompassing the signal sequence are substituted with glycine, glycine and threonine, respectively.

33. (Withdrawn) The gene of any one of claims 26 to 32 which codes for an amino acid sequence as depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2 with

deletion at its C-terminal wherein amino acid substitution is introduced.

34. (Withdrawn) The gene of any one of claims 26 to 33 which is derived from one selected from the group consisting of Fujisawa strain, Koganai strain, Tama 96 strain, SE-9 strain and Shizuoka 63 strain.

35. (Withdrawn) The gene of any one of claims 26 to 34 which has a nucleotide sequence or a nucleotide sequence with deletion of a portion of the 3'-terminal, which includes one or a combination of more than one nucleotide substitution in SEQ ID NO: 1 selected from the group consisting of (1) to (7) as described below:

(1) the 206th nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G;

(2) the 461st nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G;

(3) the 608th nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is C;

(4) the 642nd nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G;

(5) the 758th nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is C;

(6) the 833rd nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G; and

(7) the 1591st nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G.

36. (Withdrawn) The gene of any one of claims 26 to 34 which has a nucleotide sequence or a nucleotide sequence with deletion of a portion of the 3'-terminal, which includes any of nucleotide substitution in SEQ ID NO: 1 selected from the group consisting of (a) to (h) as described below:

(a) the 206th nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G;

(b) the 608th nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is C;

(c) the 642nd nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G;

(d) the 833rd nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G; and

(e) the 1591st nucleotide in the nucleotide sequence as depicted in SEQ ID NO: 1 is G;

(f) the 461st and 608th nucleotides in the nucleotide sequence as depicted in SEQ ID NO: 1 are G and C, respectively;

(g) the 642nd and 758th nucleotides in the nucleotide sequence as depicted in SEQ ID NO: 1 are G and C, respectively; and

(h) the 206th, 461st and 608th nucleotides in the nucleotide sequence as depicted in SEQ ID NO: 1 are G, G and C, respectively.

37. (Withdrawn) Use of a variant of Erysipelothrix rhusiopathiae surface protective antigen SpaA or of a shortened form thereof ΔSpaA protein in which a portion of SpaA protein is deleted, which is immunogenic and expressed in E. coli as inclusion bodies, for the preparation of a vaccine to Erysipelothrix rhusiopathiae infection.

38. (Withdrawn) The use of claim 37 wherein said variant has an amino acid sequence of SpaA or ΔSpaA protein wherein amino acid substitution is introduced.

39. (Withdrawn) The use of claim 37 or 38 wherein said variant is prepared by mutating a gene coding for SpaA or ΔSpaA protein to introduce amino acid substitution in the amino acid sequence of said SpaA or ΔSpaA protein, expressing the thus mutated gene in E. coli, and selecting among the expressed variants those that formed inclusion bodies.

40. (Withdrawn) The use of any one of claims 37 to 39 wherein said variant is prepared by the following steps (A) to (D) :

(A) introducing mutation in a gene coding for soluble Erysipelothrix rhusiopathiae surface protective antigen SpaA

or Δ SpaA protein so that amino acid substitution may be introduced;

(B) transforming E. coli cells with an expression vector containing the resulting mutated gene;

(C) selecting E. coli cells that formed insoluble inclusion bodies among the above transformed E. coli cells; and

(D) culturing the selected E. coli cells for recovery of the inclusion bodies within the cells.

41. (Withdrawn) The use of claim 40 wherein said variant is prepared by the following steps (E) to (F) subsequent to step (D) :

(E) administering the inclusion bodies or the inclusion bodies treated with a solubilizing agent to an animal sensitive to Erysipelothrix rhusiopathiae infection and then attacking said animal with a virulent strain of Erysipelothrix rhusiopathiae; and

(F) observing survival or death of the animal sensitive to Erysipelothrix rhusiopathiae to thereby assess the presence of a protective activity (immunogenicity) against Erysipelothrix rhusiopathiae infection.

42. (Withdrawn) The use of any one of claims 37 to 41 wherein said amino acid substitution in said variant is one or

a combination of more than one selected from the group consisting of (1) to (7) as described below:

- (1) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (2) the 154th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
- (3) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (4) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;
- (5) the 253rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;
- (6) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;
and
- (7) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine.

43. (Withdrawn) The use of any one of claims 37 to 41 wherein said amino acid substitution in said variant is one selected from the group consisting of (a) to (h) as described below:

- (a) the 69th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(b) the 203rd amino acid from the N-terminal encompassing the signal sequence is substituted with threonine;

(c) the 214th amino acid from the N-terminal encompassing the signal sequence is substituted with glutamine;

(d) the 278th amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(e) the 531st amino acid from the N-terminal encompassing the signal sequence is substituted with glycine;

(f) the 154th and 203rd amino acids from the N-terminal encompassing the signal sequence are substituted with glycine and threonine, respectively;

(g) the 214th and 253rd amino acids from the N-terminal encompassing the signal sequence are substituted with glutamine and threonine, respectively; and

(h) the 69th, 154th and 203rd amino acids from the N-terminal encompassing the signal sequence are substituted with glycine, glycine and threonine, respectively.

44. (Withdrawn) The use of any one of claims 37 to 43 wherein said variant has an amino acid sequence as depicted in SEQ ID NO: 2 or the sequence as depicted in SEQ ID NO: 2 with deletion at its C-terminal wherein amino acid substitution is introduced.

45. (Withdrawn) The use of any one of claims 37 to 44 wherein said variant is derived from one selected from the

group consisting of Fujisawa strain, Koganai strain, Tama 96 strain, SE-9 strain and Shizuoka 63 strain.